REMARKS

After entry of this amendment, claims 3-16 are pending. Claims 1-2 and 17-36 have been cancelled without prejudice. Applicants reserve the right to prosecute subject matter of cancelled claims in subsequent applications.

The title has been amended to be more descriptive of the invention as requested by the Examiner.

Claim 3 has been amended to recite the method of claim 6 or 7, wherein the recombinant nucleic acid molecule encoding two selectable markers further comprises one or more gene constructs of interest expressible in plastids and encoding one or more gene products of interest, and wherein the one or more gene constructs of interest are adjacent to the second selectable marker (lethal marker) on the vector. Support for these amendments is in the specification on page 17, lines 17-21.

Claim 4 has been amended to recite the method of claim 3, wherein the second selectable marker (lethal marker) and the one or more gene constructs each have different promoters functional in plastids and are expressed independently. Support for these amendments is in the specification on page 17, lines 23-26.

Claim 5 has been amended to recite the method of claim 3, wherein the second selectable marker (lethal marker) and the one or more gene constructs are organized as a operon-like polycistronic gene. Support for this amendment is in the specification on page 17, lines 26-28.

Claims 6 and 7 have been amended to be independant and to recite methods for obtaining a transplastomic plant by introducing a plastid transformation vector comprising recombinant nucleic acids encoding two selectable markers. Further, claim 6 has been amended to recite step d) obtaining a transplastomic plant by regenerating the transplastomic plant from the plant cell, wherein the transplastomic plant comprises only the second selectable marker (lethal marker). Support is in the specification on page 5 line 18 - page 6, line 2; page 17, lines 1-8, and in claims 1 and 2 as originally filed. Also the claim has been revised to state that the "first selectable marker" is also the "non-lethal marker" and that the "second selectable marker" is also the "lethal marker". Support for this amendment is in the specification on page 17, lines 1-8.

Claim 8 has been amended to recite the method of claim 7, wherein the nucleic acid molecule encoding the first selectable marker (non-lethal marker) sequence is unstably or transiently integrated into the plastome. Support for this amendment is in the specification on page 17, lines 1-8.

Claim 10 has been amended to recite the method of claim 6 or 7, wherein the first selectable marker (non-lethal marker) is an antibiotic resistance gene. Support is in the specification on page 18, lines 16-17.

Claim 12 has been amended to recite the method of claim 1, wherein the second selectable marker (lethal marker) is a herbicide tolerance gene. Support is in the specification on page 18, lines 18-19.

Claim 13 has been amended to recite the method of claim 12, wherein the herbicide tolerance gene is a mutated form of a protoporphorynogen oxidase (*ppo*) and the lethal selective compound is butafenacil or Formula XVII. Support is in the specification on page 20, lines 32-33 and page 24, lines 31-34.

Claim 14 has been amended to recite the method of claim 6, wherein the plastid transformation vector comprises SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:19. This amendment was to correct antecedent basis for the plastid transformation vector.

Claim 15 has been amended to recite the method of claim 7, wherein the plastid transformation vector comprises SEQ ID NO:3. This amendment was to correct antecedent basis for the plastid transformation vector.

Claim 16 has been amended to recite the method of claim 6 or 7, wherein the plant is tobacco, tomato, petunia, potato, brassica spp. , safflower, or lemna. This amendment makes the claim dependent upon independent claims 6 or 7 instead of cancelled claim 1.

No new matter has been added by these amendments.

Claim Objections

Claims 14 and 15 have been objected to as having an improper article before the word "plastid". The claims have been amended to delete the article "a" and replaced it with "the".

Claim Rejections under 35 USC § 112

Claims 1-16 are rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention.

Claims 1 and 2 have been canceled, and claims 3-16 have been amended to more particularly point out and distinctly claim the invention. Applicants submit these amendments overcome the rejections and request their withdrawal.

Claim Rejections under 35 USC § 102

Claims 1-12 and 16 are rejected under 35 USC § 102(e) as allegedly being anticipated by Staub et al. (US Patent Publication No. 2002/0042934).

Applicants respectfully disagree with this rejection.

The legal test for anticipation under 35 U.S.C. § 102 requires that each and every element of the claimed invention be disclosed in a prior art reference in a manner sufficient to enable one skilled in the art to reduce the invention to practice, thus placing the public in possession of the invention. W.L. Gore Assoc. v. Garlock, Inc., 721 F.2d 1540, 1554 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1994); In re Donohue, 766 F.2d 531 (Fed. Cir. 1985). Anticipation under 35 U.S.C. § 102 requires identity of invention. Scripps Clinical & Research Fdn. v. Genentech, Inc., 927 F.2d 1565 (Fed. Cir. 1991).

Claims 1-2 have been cancelled without prejudice, thereby making this rejection moot.

Claims 6-7 have been amended to be independent and more descriptive of the invention. The presently claimed invention is for methods to obtain transplastomic plants that comprise a plastid lethal selectable marker gene and in which the non-lethal selectable marker is no longer present. There are two ways this can be accomplished: 1) by inserting the non-lethal selectable marker in the backbone of the vector or 2) inserting the non-lethal selectable marker between non-essential plastid nucleotide sequences.

The first is recited in claim 6 where the non-lethal selectable marker is in the backbone of the vector as in vector pEPPOt. See Figures 1 and 4 and the description on pages 9-10. The result is "the stable integration of the lethal marker gene (ppo) in the plastid genome and the complete excition of the non-lethal marker gene (aadA) with the vector backbone sequences."

The second method is recited in claim 7 in which the non-lethal selectable marker is inserted within an essential plastid sequence and the lethal selectable marker is inserted within a non-essential plastid sequence as in vector pNY2C (Figure 3). In this example, the aadA gene is inserted withing the ycf2 essential gene and the ppo gene is inserted into the intergenic region between plastid genes ndhB and trnL. Figure 6 shows the results after selection on the non-lethal and lethal compounds. These figures are described on pages 9-10. Upon withdrawal of selection on the non-lethal compound (spectinomycin) the non-lethal gene within the essential gene will be lost. Selection on the lethal compound (butafenacil) maintains the lethal selectable marker within the non-essential plastid sequences.

The presently claimed invention is designed to ensure that the non-lethal selectable marker is "lost" and recombines out of the plastid genome. This differs from the method of Staub et al. (US 2002/0042934) which obtains only a fraction of the transformed plant tissue containing only the lethal selectable marker. As described in paragraph [0073] "The transplastomic plant cells thus arising on phase two selection will yield transplastomic plants containing plastids having either a single construct insert providing expression of the gene providing tolerance to a plastid lethal compountd or both introduced constructs providing expression of the genes providing tolerance to a plastid non-lethal and plastid lethal compound. Transplastomic plants can then be analyzed, used for additional rounds of plant regeneration to obtain homoplasmy, and, if desired, selected for the single construct insertion."

Further, as demonstrated in Staub et al., in Example 3, paragraph [0128] "The data indicates a frequency of aadA + CP4 cotransformation in phase 1 as 20%; in phase 2, half of these (50%) segregate genomes containing the CP4 gene alone (CP4 + segregants). Therefore, the overall frequency of segregated CP4+genomes is 10% of the total transformants using the phased selection scheme."

Therefore, the above comments and amendments overcome this rejection, and Applicants request its withdrawal.

Claim Rejections under 35 USC § 103

Claims 1-13 and 16 are rejected under 35 USC § 103(a) as allegedly being unpatentable over Staub et al. (US Patent Publ. 2002/0042934) in view of Fonee-Pfister et al. (WO 01/68826).

Applicants respectfully disagree for the reasons given above. The presently claimed invention was not described, much less taught, by the combination of Staub in view of Fonee-Pfister since the present method assures loss of the non-lethal selectable marker to obtain transplastomic plants comprising only the lethal selectable marker after the two step selection process.

The above remarks and amendments overcome this rejection, and Applicants respectfully request its withdrawal.

Should the Examiner have any questions, please contact the Attorney of Record below by telephone.

Respectfully submitted,

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